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Dean L. Engelhardt et al.

Serial No.: 08/486,066

Filed: June 7, 1995

Page 2 (Supplemental Response to Applicants' April 29, 1997 Amendment  
Under 37 C.F.R. §1.115 - May 28, 1997)

### **REMARKS**

In their April 29, 1997 Amendment Under 37 C.F.R. §1.115 (page 6, fourth paragraph under REMARKS), Applicants indicated that they were awaiting receipt of a Communication Under Rule 51(4) EPC from the European Patent Office in connection with a corresponding European patent application. As the Examiner knows, the Rule 51(4) EPC Communication is the equivalent of a Notice of Allowance in the U.S. Patent and Trademark Office.

By this Supplemental Response, Applicants and their attorney are submitting correspondence and documents that were recently sent by their European associates who are handling the prosecution of their corresponding European Patent Application No. 88 10 4963.9-2105 (European Patent Publication No. 0 286 898 A2). Among these submitted papers is their European associates' April 29, 1997 letter (copy attached as Exhibit A), the Communication Under Rule 51(4) EPC dated March 12, 1997 (Exhibit B), and the claims to be granted or the allowed claims, 1-18 (Exhibit C).

Applicants respectfully request that the Examiner consider these developments in the EPO at the same time that he examines this application further. In particular, the fact that the European examiner accepted language in the independent claim where "S is a sugar moiety" should be considered when weighing Applicants' remarks in response to the objection and rejection under 35 U.S.C. §112, first paragraph.

Early and favorable action on pending claims 238-297 and 299-338 is respectfully requested.

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Dean L. Engelhardt et al.

Serial No.: 08/486,066

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Page 3 (Supplemental Response to Applicants' April 29, 1997 Amendment  
Under 37 C.F.R. § 1.115 - May 28, 1997)

### **SUMMARY AND CONCLUSIONS**

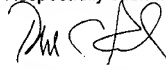
By this Supplemental Response, claims 238-297 and 299-338 are presented for further examination. No claims have been added, canceled or amended herein.

No fee is deemed necessary in connection with the filing of this Supplemental Response. If any fee is deemed necessary, however, authorization is hereby given to charge the amount of any such fee to Deposit Account No. 05-1135.

If it would be helpful to expediting prosecution of this application, Applicants' undersigned attorney may be contacted by telephone at 212-583-0100 during normal business hours.

Early allowance of the pending claims is respectfully requested.

Respectfully submitted,



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# **EXHIBIT A**



## Isolation and Properties of a *Pseudomonas acidovorans* Bacteriophage

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(Accepted 22 August 1969)

### SUMMARY

The lytic bacteriophage  $\phi$ W-14 was isolated from sewage using *Pseudomonas acidovorans* no. 14 as host. The phage had an icosahedral head some 85 nm. in diameter and a contractile tail some 140 nm. long.  $\phi$ W-14 formed plaques on only a few strains of *P. acidovorans*. The phage gave biphasic adsorption kinetics, with an adsorption constant of  $1.9 \times 10^{-9}$  ml./min. The latent period was 60 min. and the burst size was about 300. The burst size was dependent upon culture age. The  $k_{in}$  for inactivation of  $\phi$ W-14 was  $4.35 \text{ min.}^{-1}$ . *P. acidovorans* was shown to possess a potent photoreactivating system. The heat of inactivation of  $\phi$ W-14 was calculated to be 75,700 cal./mole. The phage gave biphasic thermal inactivation kinetics at 55° and 60° but not at 65°. The phage mutated spontaneously to a different plaque type. This mutation affected the adsorptive properties, the thermal sensitivity and the burst size of the phage.

### INTRODUCTION

There have been relatively few studies of the bacteriophages of non-fluorescent strains of *Pseudomonas*. Phages have been reported for *P. fragi* (Roberts & Doetsch, 1966), *P. stutzeri* (Espejo & Canelo, 1968) and *P. putrefaciens* (Levin & Delisle, 1969). The present report describes the isolation and the properties of a *P. acidovorans* phage.

### METHODS

**Bacteria.** These are listed in Table 1. They were maintained at 4° in standard minimal case stabs with 0.5% yeast extract (Stanier, Palleroni & Doudoroff, 1966), and were transferred once a month.

**Media.** All media were made up in distilled water. The bacteria were usually grown in mannitol broth (MB) containing, in g./l.: tryptone, 10; yeast extract, 5; NaCl, 5; mannitol, 1. The pH was adjusted to 6.5 before autoclaving. The medium used for adsorption studies (AM) contained, in g./l.: tryptone, 1; yeast extract, 0.5; mannitol, 2.

**Growth of bacteria.** Unless stated otherwise, cultures were incubated in a Metalyte G 77 shaker water bath (New Brunswick Scientific Co., New Brunswick, N.J.) at 30° and 250 rev./min.

**Titration of phage.** Phage was assayed and plaque morphology examined by the agar layer technique (Adams, 1959). The top and bottom layers consisted of MB containing 0.6 and 1.5% agar, respectively.

**Isolation of phage.** A sample of activated sludge from the Greater Vancouver

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Sewage Processing Plant was centrifuged at 20,000 *g* for 5 min. A 10 ml. sample of the supernatant fluid and 2 drops of an overnight culture of *P. acidovorans* no. 14 were added to 10 ml. of double strength MB in a 250 ml. Erlenmeyer flask. After incubation for 48 hr without shaking, the culture was centrifuged at 6000 *g* for 10 min. A sample of the supernatant was diluted and plated with *P. acidovorans* no. 14. A well-isolated plaque was picked with a sterile wire and the phage purified by serial single plaque isolations. The phage was given the designation  $\phi$ W-14.

Table 1. Sources of bacteria

Organism	Strain	Source
<i>P. acidovorans</i>	Nos. 14, 29, 114, 146	Dr R. Y. Stanier, Department of Bacteriology University of California, Berkeley
	AK-11	Norleucine enrichment. A. Kropinski
	15666, 15667 15668	Dr E. F. Lessel Curator of Bacteria, American Type Culture Collection
<i>P. testosteroni</i>	Nos. 78, 138 11996	Dr R. Y. Stanier Dr E. F. Lessel
<i>P. mucidolens</i>	ATCC 4687	Culture Collection Department of Microbiology University of British Columbia Vancouver
<i>P. putrefaciens</i>	HAMMER	Department of Microbiology, U.B.C.
<i>P. ovalis</i>	ATCC 950	Department of Microbiology, U.B.C.
<i>P. taetrolens</i>	ATCC 4683	Department of Microbiology, U.B.C.
<i>P. synxantha</i>	ATCC 796	Department of Microbiology, U.B.C.
<i>P. convexa</i>	ATCC 795	Department of Microbiology, U.B.C.
<i>P. aeruginosa</i>	ATCC 9027	Department of Microbiology, U.B.C.
<i>P. aeruginosa</i>	ATCC 9721	Department of Microbiology, U.B.C.
<i>P. fragi</i>	ATCC 4975	Department of Microbiology, U.B.C.

**Preparation of high titre lysates.** A number of 500 ml. Erlenmeyer flasks each containing 150–200 ml. of MB were inoculated with 10 ml. of an overnight culture of *P. acidovorans* no. 29 and incubated until the cultures reached an optical density at 650 nm. of 1.5 to 2.0, equivalent to  $1.0$  to  $1.3 \times 10^8$  cells/ml. Phage  $\phi$ W-14 was added to give a multiplicity of infection (m.o.i.) of about 1, and incubation continued for a further 6 hr. The culture did not clear under these conditions. The lysate was freed of whole cells and debris by centrifugation at 20,000 *g* for 5 min. This method routinely yielded lysates with titres of  $1$  to  $3 \times 10^{11}$  p.f.u./ml.

**Host range.** The host ranges of the phages were determined by spotting drops of high titre lysates (c.  $1 \times 10^{12}$  p.f.u./ml.) on overlays containing the various organisms, and examining for lysis after overnight incubation at 30°.

**One-step growth experiment.** The latent period and average burst size for *P. acidovorans* no. 29 were determined by the one-step growth method (Adams, 1959). The infected culture, in MB, was incubated in the shaker water bath at 30° and 250 rev./min. to allow for adsorption.

**Electron microscopy.** Preparations of phage  $\phi$ W-14 were stained with 2% phosphotungstic acid, pH 7.2. The grids were allowed to air dry before being examined with a Philips EM-200 electron microscope at an operating voltage of 60 kv. Magnifications, before printing, ranged from 15,000 to 27,800.

**Kinetics of adsorption.** Adsorption to *P. acidovorans* no. 29 was measured by assaying the unadsorbed phage after removing the bacteria by centrifugation (Adams,

1959). The phage was added at a m.o.i. of 0.01 to cultures at a cell density of  $10^8$ /ml., in either MB or AM, incubated in the shaker water bath at 30° and 250 rev./min.

**Cell lysis.** Lysis was followed in two ways. In the turbidimetric method, phage was added at a m.o.i. of 5 to 10 ml. of a log. phase MB culture of *P. acidovorans* no. 29 in a 125 ml. sidearm flask. The turbidity of the culture was read at 30 min. intervals in a Klett Summerson Photoelectric colorimeter (Klett Mfg. Co., New York, N.Y.) equipped with a 540 filter. In the one-step growth method, phage was added to 10 ml. of a log phase MB culture ( $1 \times 10^8$  cells/ml.) at m.o.i. of 5. After allowing 10 min. for adsorption, more phage was added, again at a m.o.i. of 5. After a further 10 min., the culture was diluted and plated as in the one-step growth experiment.

**Thermal inactivation of phage.** Large tubes (2.2 x 20 cm.) containing 10 ml. of MB were placed in water baths at 50°, 55°, 60° and 65°. After 10 min. for temperature equilibration, phage suspension, generally less than 0.2 ml., was added to each tube to give  $1.5$  to  $3.5 \times 10^3$  p.f.u./ml. (except the tube at 65°, in which the initial titre was  $2 \times 10^4$  p.f.u./ml.). At regular intervals, 0.5 ml. samples were removed to small test tubes chilled in an ice-bucket. All the samples were plated at the end of the experiment. The rate constants for thermal inactivation were calculated using the equation of Pollard (1953). The activation energies were calculated using the equation given by Neilands & Stumpf (1958), applying the corrective equation given by Dixon & Webb, (1964).

**Sonic sensitivity of phage.** Quadruplicate 2.8 ml. samples of phage each containing  $1 \times 10^6$  p.f.u./ml. were subjected to 30 sec. bursts of acoustic energy from a Biosonic II (Bronwill Scientific, Rochester, N.Y.), equipped with a needle probe. The frequency of ultrasonic output of this instrument is 20 kcyc./sec.  $\pm$  400 cyc./sec. A setting of 70 was used throughout, being equivalent to 87.5 w. The samples were chilled in ice-water prior to and during sonication to reduce heat denaturation. Then they were diluted and 0.1 ml. samples plated.

**pH sensitivity of phage.** Samples of MB medium were adjusted to pH 2-12 by the addition of 1M-HCl or 1M-NaOH, and 4.5 ml. amounts were added to test tubes in an ice-water bath. After 15 min., 0.5 ml. of a phage preparation diluted to  $3 \times 10^4$  p.f.u./ml. was added to each and the tube shaken vigorously. After a further 30 min., 0.1 ml. samples were plated from each tube.

**Sensitivity of phage to u.v. light.** One millilitre samples of a mixture of coliphage T1 and  $\phi$ W-14a<sup>+</sup> in MB (each at  $1 \times 10^8$  p.f.u./ml.) were placed in disposable plastic Petri dishes (Millipore Filter Corp., Bedford, Mass.) of 5.0 cm. diameter and irradiated at a distance of 50 cm. with a General Electric 15 w germicidal lamp (principle wavelength at 2537 Å) with continuous stirring by a magnetic stir-bar and stirrer. The irradiated samples were then diluted and plated under yellow, nonphotoreactivating light with *P. acidovorans* no. 29 for  $\phi$ W-14a<sup>+</sup> and *Escherichia coli* B for T1. The plates were incubated at 30° and 37°, for  $\phi$ W-14a<sup>+</sup> and T1 respectively, in lightproof boxes. The rate constants for inactivation were calculated from the exponential region of the graphed results using the equation of Pollard (1953).

**Photoreactivation of u.v. irradiated phage.** The u.v.-irradiated samples of phage were plated under white light, and the uninverted plates were incubated overnight 30 cm. from a lamp fitted with twin Westinghouse 15 w Cool White or General Electric 15 w Daylight fluorescent bulbs (principle wavelength approximately 5700 Å in both cases). The photoreactivable sector was calculated using the equation of Dulbecco (1950).

*Morphology*

## RESULTS

$\phi$ W-14 exhibited a regular icosahedral head about 85 nm. across, with a tail some 20 nm. in diameter and 140 nm. long (Fig. 1*a*). Subunits could be seen in the tails of some of the particles (Fig. 1*a*). A baseplate at the end of the tail appeared to carry

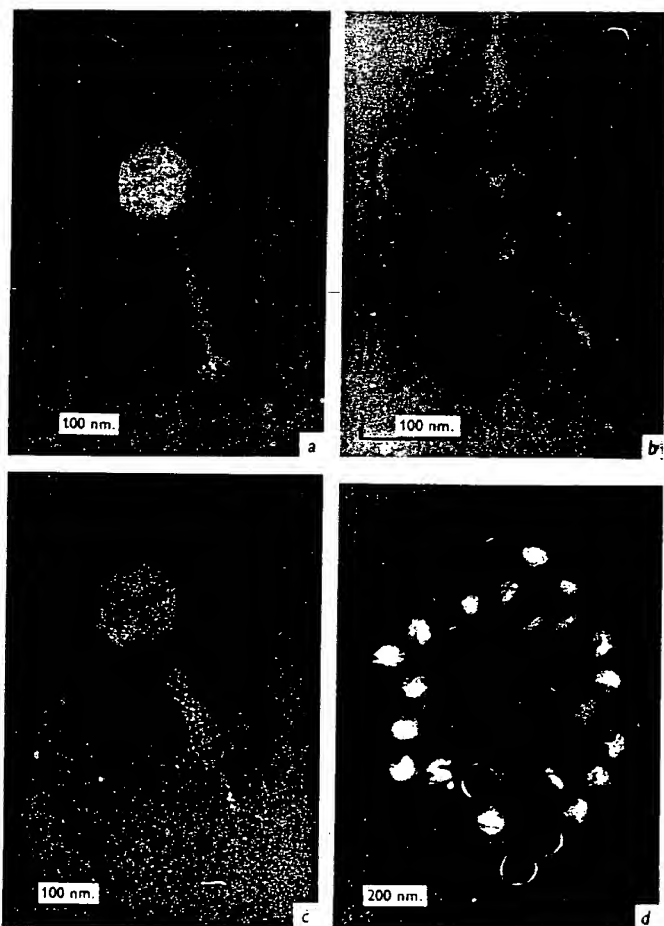


Fig. 1. (a)  $\phi$ W-14. (b)  $\phi$ W-14, tail detail and empty heads. (c)  $\phi$ W-14 with contracted sheath. (d) Aggregate of  $\phi$ W-14 particles.



pin-like structures (Fig. 1*b*). A number of empty heads were observed which retained largely the angular shape of the complete head (Fig. 1*b*). A few complete particles with empty heads and a great many free tails were observed in the preparation (Fig. 1*b, d*). The addition of sodium perchlorate, buffered at pH 7 to 9, to a concentration of approximately 0.025 M (Freifelder, 1966), caused contraction of the tail sheath of some particles (Fig. 1*c*). Some of the contracted particles seemed to have a collar at the juncture of the head and tail. Contraction of the sheath exposed a tail core, and a skirt of projections, originating in the region of the former baseplate, fanned out around the exposed core (Fig. 1*c*).

Table 2. *Host-range of  $\phi W$ -14a<sup>-</sup> and derivatives*

Organism	Lysis by		Organism	Lysis by	
	$\phi W$ -14a <sup>-</sup>	$\phi W$ -14a		$\phi W$ -14a <sup>-</sup>	$\phi W$ -14a
<i>P. acidovorans</i> no. 29	+	+	<i>P. testosteroni</i> no. 138	-	nd
<i>P. acidovorans</i> no. 14	+	+	<i>P. testosteroni</i> 11996	-	nd
<i>P. acidovorans</i> no. 114	-	-	<i>P. aeruginosa</i> 9027	-	nd
<i>P. acidovorans</i> no. 146	-	-	<i>P. aeruginosa</i> 9721	-	nd
<i>P. acidovorans</i> AK-11	+	+	<i>P. ovalis</i> 950	-	nd
<i>P. acidovorans</i> 15666	-	-	<i>P. fragi</i> 4975	-	nd
<i>P. acidovorans</i> 15667	-	-	<i>P. convexa</i> 795	-	nd
<i>P. acidovorans</i> 15668	+	+	<i>E. coli</i> k 12	-	nd
<i>P. testosteroni</i> no. 78	-	nd*	<i>E. coli</i> B	-	nd

\*nd = not done.

Phage  $\phi W$ -14 showed a marked tendency to aggregate (Fig. 1*d*). No bacterial debris appeared to be present to account for this aggregation.

#### Plaque morphology

When first isolated,  $\phi W$ -14 formed small plaques, about 2 mm. in diameter, with very small, clear centres and turbid haloes, on *Pseudomonas acidovorans* no. 14. These were designated a<sup>-</sup>. Later, mutants appeared, designated a, producing slightly smaller plaques with wide, clear centres and indistinct haloes. The a type appeared in lysates prepared with *P. acidovorans* no. 14, but not in those prepared with *P. acidovorans* no. 29. Because the a type gave a higher burst size, difficulty was encountered in preparing a high titre lysate of predominantly the a<sup>-</sup> type when *P. acidovorans* no. 14. was used as the host. The a type appeared to be more stable than the a<sup>-</sup> type, since reversion was rarely, if ever, observed. The plaques formed on *P. acidovorans* AK-11 were small and indistinct, so that the efficiency of plating (e.o.p.) could not be determined accurately with this strain.

#### Host range

$\phi W$ -14 showed a limited host range, lysing only four of seven different strains of *Pseudomonas acidovorans* (Table 2). It did not lyse any strains of the related organism, *P. testosteroni*.  $\phi W$ -14a<sup>-</sup> and  $\phi W$ -14a showed identical host ranges, and on each indicator the plaques of the a<sup>-</sup> type were haloed and those of the a type were clear. The e.o.p. was 2 to 3 times higher on *P. acidovorans* no. 29 than on *P. acidovorans* no. 14 with both the a<sup>-</sup> type and the a type prepared on either host strain.

### Kinetics of adsorption

In MB, the concentration of unadsorbed phage decreased exponentially with first order kinetics, with adsorption constants of  $1.9 \times 10^{-9}$  ml./min. and  $4.2 \times 10^{-9}$  ml./min. for  $\phi W-14a^-$  and  $\phi W-14a$ , respectively. Only 60% of  $\phi W-14a^-$  appeared to adsorb, while the rate constant for  $\phi W-14a$  decreased to  $1.5 \times 10^{-9}$  ml./min. after 90% of it had adsorbed. The adsorption rates were the same at 30° and 0°. In low salt medium (AM) the rate constant for  $\phi W-14a^-$  increased to  $3.0 \times 10^{-9}$  ml./min., with 90% of it adsorbing under these conditions, whereas the rate for  $\phi W-14a$  was the same in AM and MB. The rate constant for  $\phi W-14a^-$  was decreased to  $7.3 \times 10^{-10}$  ml./min. when the phage-host mixture was incubated at 50 rev./min. rather than the normal 250 rev./min.

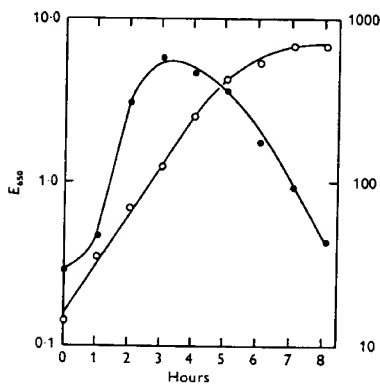


Fig. 2

Fig. 2. Effect of cell age on phage development as measured by the one-step growth method. ○—○, growth of *P. acidovorans* no. 29 at 30° measured in  $E_{650}$  units/ml. ●—●, average burst size from cell samples taken at specified times and infected with  $\phi W-14a^-$ .

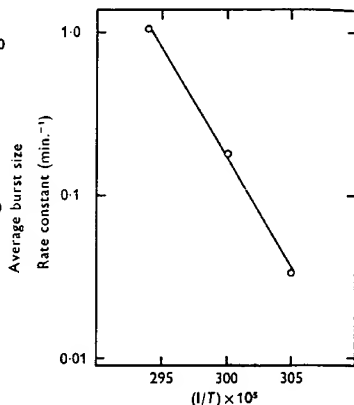


Fig. 3

Fig. 3. Arrhenius plot of the thermal inactivation of  $\phi W-14a^-$ .

### One-step growth experiment

Most of the one-step growth experiments were carried out with *Pseudomonas acidovorans* no. 29 because of the slow adsorption of  $\phi W-14$  to *P. acidovorans* no. 14.

The latent period for  $\phi W-14a^-$  was about 60 min. and the rise period about 40 min. The burst size, averaged over six experiments, was 300, with a range from 214 to 470. These experiments were performed with cells in the logarithmic phase of growth, i.e. at a density of about  $10^8$  cells/ml., diluted to  $10^6$  cells/ml. before infection. Subsequently, it was found that the burst size was markedly dependent upon the age of culture (Fig. 2), ranging from 30 for stationary phase cells to about 600 for late log. phase cells. A similar response was observed with *Pseudomonas acidovorans* no. 14. This was not due to effects on the adsorption of the phage, since the capacity of the cells to adsorb the phage remained constant over the growth curve.

The latent and rise periods for  $\phi W-14a$  were the same length as those for  $\phi W-14a^-$ , but the burst size was 50 % greater.

Attempts to lyse infected cells prematurely with chloroform or lysozyme were unsuccessful.

#### Lysis inhibition

The turbidity of a culture of *Pseudomonas acidovorans* no. 29 decreased markedly 30 min. after infection with  $\phi W-14a$ , and then remained constant. Following infection of the same strain with  $\phi W-14a^-$ , there was a slight decrease in the turbidity of the culture after 30 min., followed by a gradual increase during the next 3 hr. Thus, a culture infected with  $\phi W-14a$  appeared to be lysis inhibited. However, attempts to demonstrate lysis inhibition by superinfection with  $\phi W-14a^-$  were unsuccessful. Surprisingly, superinfection decreased the burst size, and it was found subsequently that use of a m.o.i. greater than about 5 led to a reduction in burst size with both  $\phi W-14a^-$  and  $\phi W-14a$ .

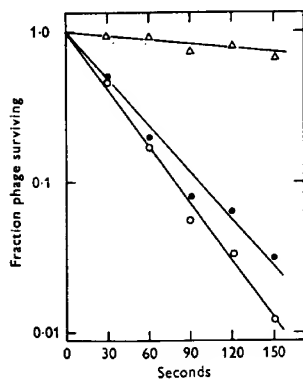


Fig. 4

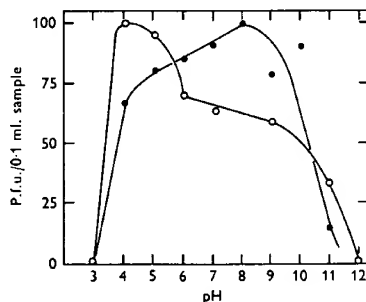


Fig. 5

Fig. 4. Sonic sensitivity of coliphage S13, T1 and  $\phi W-14a^+$ : ( $\Delta-\Delta$ ), S13: ( $\bullet-\bullet$ ), T1: ( $\circ-\circ$ ),  $\phi W-14a^-$ .

Fig. 5. pH Inactivation of  $\phi W-14a^+$  ( $\bullet-\bullet$ ) and  $\phi W-14a$  ( $\circ-\circ$ ).

#### Thermal inactivation

$\phi W-14a^+$  was essentially stable at 50° in MB. At temperatures above this, the rate constants for inactivation were 0.033 min.<sup>-1</sup>, 0.18 min.<sup>-1</sup> and 1.06 min.<sup>-1</sup> at 55°, 60° and 65°, respectively. At 55° and 60°, the inactivation curve was biphasic, with some 35 % of the particles having some degree of resistance. The resistant fraction appeared to be stable at 55°, but was inactivated slowly at 60°. Using the initial parts of the curves, the heat of activation of  $\phi W-14a^+$  was calculated to be 75,700 cal./mol. (Fig. 3).

$\phi W-14a$  was slightly more thermolabile than  $\phi W-14a^+$ , the activation energy being 62,500 cal./mole. It showed biphasic inactivation at 55° but not at 60°.

#### Sonic sensitivity

$\phi$ W-14a<sup>-</sup>, with a  $k$ , value of 1.74 min.<sup>-1</sup>, was slightly more sensitive to sonication than coliphage T1, which gave a  $k$ , value of 1.45 min.<sup>-1</sup> (Fig. 4). Coliphage S13 was much less sensitive than phage T1, giving a  $k$ , value of 0.12 min.<sup>-1</sup> (Fig. 3).

#### pH sensitivity

Both  $\phi$ W-14a<sup>-</sup> and  $\phi$ W-14a were relatively stable between pH 5 and pH 9 (Fig. 4). However, since phage lysates were prepared and stored at pH 6.5 to 7.0, it appeared that  $\phi$ W-14a was activated slightly at pH 4-5 (Fig. 5).

#### Sensitivity to ultraviolet light

Multihit kinetics were observed for the inactivation of  $\phi$ W-14a<sup>-</sup> and coliphage T1. The rate constants ( $k_{uv}$ ), calculated from the exponential regions of the curves, were 0.60 min.<sup>-1</sup> for T1, and 4.35 min.<sup>-1</sup> for  $\phi$ W-14a<sup>-</sup>. The lethal effects of u.v. light were reversed to a considerable extent by irradiation of the overlay plates with white light. Photoreactivable sectors of 0.35 and 0.71 were calculated for T1 and  $\phi$ W-14a<sup>+</sup>, respectively.

#### DISCUSSION

$\phi$ W-14 falls into Bradley's morphological classification group A, which contains all phages having contractile tails (Bradley, 1967). Morphologically it resembles other *Pseudomonas* phages, but it is considerably larger than those reported previously, the heads of which range from 50 to 60 nm. in diameter (Lee & Boezi, 1966; Bradley, 1967; Olsen, Metcalf & Todd, 1968). The aggregation of  $\phi$ W-14 particles seen in some of the electron micrographs appeared not to involve adsorption to bacterial debris.

$\phi$ W-14 has a very limited host range, with the e.o.p. on the susceptible hosts varying over only a two- to threefold range.

The biphasic adsorption kinetics observed with  $\phi$ W-14 are unusual, although phage 7v of *P. aeruginosa* (Feary, Fisher & Fisher, 1964) and phage  $\chi$  of *E. coli* (Schade & Adler, 1967) yield significant numbers of slowly adsorbing particles. The poor adsorption under static conditions remains to be explained.

Compared with other *Pseudomonas* phages,  $\phi$ W-14 has a high burst size. Although it has a long latent period, several *P. aeruginosa* phages have been reported recently to have latent periods of 65 to 70 min. (O'Callaghan, O'Mara & Grogan, 1969). The decrease in burst size following superinfection is unusual, and cannot be explained at present. Cells of *Shigella sonnei* gave smaller burst sizes following infection with a large excess of coliphages T4 and T7 (Barry & Goebel, 1951).

Biphasic thermal inactivation curves, like those obtained for  $\phi$ W-14, though quite common amongst animal viruses, have not been reported often for bacteriophages. Some 35% of *P. aeruginosa* phage 7m particles appeared to be more heat resistant than the remainder of the population (Feary *et al.* 1964). The proportion of heat resistant  $\phi$ W-14 particles corresponded to the proportion of slowly adsorbing particles, so that the change from  $a^-$  to  $a$  may involve the adsorption structures of the phage tail.

We thank Dr M. Weintraub of the Research Station, Canada Department of Agriculture, North West Marine Drive, Vancouver, for making available to us the facilities of his laboratory for the preparation of the electron micrographs.

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(Received 17 July 1969)

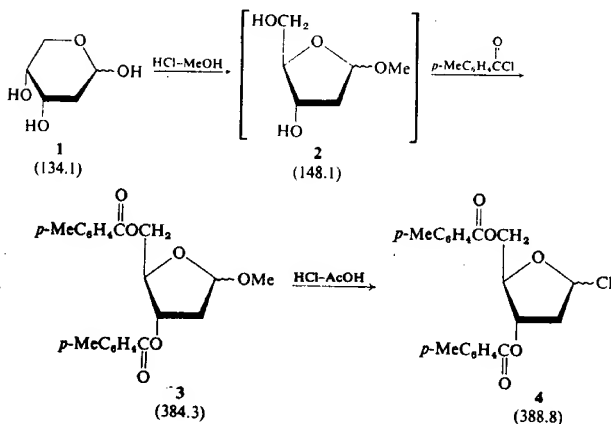
# **EXHIBIT C**

**[158] 2-Deoxy-3,5-di-*O*-*p*-toluoyl-D-erythro-pentosyl  
Chloride**

*Preparation of a Crystalline O-Acyl-2-deoxypentofuranosyl Halide*

**CLARITA C. BHAT**

DEPARTMENT OF CHEMISTRY, GEORGETOWN UNIVERSITY,  
WASHINGTON, D.C. 20007



**INTRODUCTION**

The title compound (4) is a relatively stable, crystalline *O*-acylglycosyl halide, and has utility in the direct synthesis of 2-deoxy-D-erythro-pentofuranosyl nucleosides (2'-deoxyribonucleosides).

**PROCEDURE**

**Methyl 2-Deoxy-3,5-di-*O*-*p*-toluoyl-D-erythro-pentoside<sup>1</sup> (3)**

To a solution of 13.6 g. (0.1 mole) of 2-deoxy-D-erythro-pentose (1) in 243 ml. of methanol is added 27 ml. of a 1% solution of hydrogen chloride in methanol. The mixture is kept in a stoppered flask for 12-15 min.,<sup>a</sup>

<sup>a</sup> The optimal yield of methyl 2-deoxy-D-erythro-pentofuranoside (2) is obtained in this time interval.

after which the reaction is stopped by adding, with vigorous stirring, 5 g. of silver carbonate. After filtration, the clear solution is evaporated to a sirup under diminished pressure.

Residual methanol is removed by repeated evaporations under diminished pressure with small volumes of dry pyridine, the sirup **2** is dissolved in 80 ml. of pyridine, and the solution is cooled in an ice bath. To this solution is quickly added 34 g. (0.22 mole) of *p*-toluoyl chloride. The reaction mixture is stirred at 0° for 1 hr., gradually warmed to 40–50° and kept either at this temperature for 2 hr., or at room temperature overnight. The mixture is poured, with stirring, onto 300 ml. of crushed ice, and when the ice has melted, the mixture is extracted with three 150-ml. portions of ether. The ether extracts are combined and successively washed with water, dilute sulfuric acid, and aqueous sodium hydrogen carbonate. Evaporation of the extract under diminished pressure yields a yellowish sirup, which may be crystallized at this point to give a mixture of anomers of methyl 2-deoxy-3,5-di-*O-p*-toluoyl-D-*erythro*-pentoside (**3**); yield 26.9 g. (70%).

#### 2-Deoxy-3,5-di-*O-p*-toluoyl-D-*erythro*-pentosyl Chloride (**4**)

For the direct preparation of the chloride, the above sirup (**3**) is dissolved in 40 ml. of glacial acetic acid, and to this solution is added 80 ml. of glacial acetic acid presaturated with dry hydrogen chloride at 10°. Hydrogen chloride is passed into the solution for 10 min., whereupon the chloride (**2**) solidifies, forming a thick, crystalline mass. After 30 min., the crystals are filtered off by suction, and thoroughly washed with dry ether. The crystals are then suspended in dry ether, filtered off, and stored in a vacuum desiccator containing soda-lime and phosphorus pentaoxide; yield, 27.5 g. (70%, based on **1**), m.p. 109° (dec.),  $[\alpha]_D^{25} + 108 \rightarrow +65^\circ$  (90 min., *c* 1.0, *N,N*-dimethylformamide). Compound **2** is stable for weeks when stored over soda-lime and phosphorus pentaoxide, but decomposes in a few hours if exposed to moisture. Small amounts of the chloride (**4**) may be recrystallized from toluene or carbon tetrachloride.

#### REFERENCE

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# Synthetic Procedures in Nucleic Acid Chemistry

VOLUME 1

*Preparation of Purines, Pyrimidines,  
Nucleosides, and Nucleotides*

EDITED BY

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DEPARTMENT OF BIO-ORGANIC CHEMISTRY  
GULF SOUTH RESEARCH INSTITUTE  
NEW IBERIA, LOUISIANA

AND

**R. STUART TIPSON**

NATIONAL BUREAU OF STANDARDS  
WASHINGTON, D.C.

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SCIENCE

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# **EXHIBIT D**

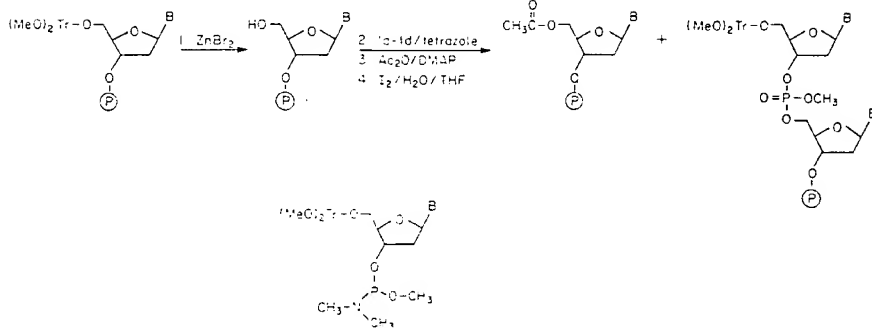
## SECOND ANNUAL CONGRESS FOR RECOMBINANT DNA RESEARCH

THE ROLE OF SYNTHETIC DNA IN RECOMBINANT DNA RESEARCH.  
Marvin H. Caruthers. Department of Chemistry, University  
of Colorado, Boulder, CO. 80309.

An important and integral part of any program involving recombinant DNA is the ability to manipulate and modify DNA sequences in any predetermined manner. This task can best be performed by DNA synthesis. During the past four years, we have developed a new, extremely rapid, high yield method for chemically synthesizing deoxyoligonucleotides. This methodology will be described. Additionally, several biochemical investigations utilizing this chemistry will be outlined.

The synthesis begins by derivatizing high performance liquid chromatography grade silica gel to contain an appropriately protected nucleoside. The deoxynucleoside is linked through the 3'-hydroxyl to a carboxylic acid functional group attached covalently to silica gel. Chemical steps for the addition of one nucleotide to the support are illustrated in the figure. The four steps are the following:

### SYNTHESIS OF A DINUCLEOTIDE



Steps in the Synthesis of a Dinucleotide. P, a silica gel polymeric support;  $(\text{MeO})_2\text{Tr}$ , di-*p*-anisylphenylmethyl; DMAP, dimethylaminopyridine.

(Page 1 of 2)

## SECOND ANNUAL CONGRESS FOR RECOMBINANT DNA RESEARCH

(Caruthers cont.)

(1) detritylation using  $\text{ZnBr}_2$  in nitromethane/methanol (5 min); (2) condensation of a 5'-di-p-anisylphenylmethyl deoxynucleoside 3'-methoxy-N,N-dimethylamino phosphine with the support bound nucleoside (5 min); (3) blocking unreacted support bound nucleoside hydroxyl groups with acetic anhydride (5 min); (4) oxidation of the phosphite to the phosphate with I (2 min). Completed deoxyoligonucleotides are isolated by first sequentially treating with thiophenol, t-butylamine, ammonium hydroxide, and 80% acetic acid and then purifying to homogeneity by electrophoresis on polyacrylamide gels. The method is extremely fast (approximately 30 min per cycle), yields in excess of 95% per condensation are obtained, and isolation of the final product is a simple one step purification.

Synthetic deoxyoligonucleotides can then be joined to form biochemically active DNA duplexes. Of particular recent interest to this laboratory have been studies involving sequence modified *E. coli* promoters, operators, and ribosome binding sites. Various other projects have focused on the construction of genes coding for proteins such as the type I interferons.

# DNA

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## GENERAL INFORMATION

*DNA deals with the application of recombinant DNA technology to problems of gene structure and function, regulation of gene expression, cell biology, developmental biology, gene transfer, and protein synthesis.*

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# DNA

VOLUME 1

NUMBER 2

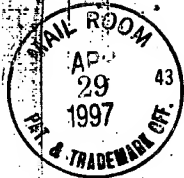
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# **EXHIBIT 1**



IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

April 16, 1991  
New York, New York

Honorable Commissioner of  
Patents and Trademarks  
Washington, D.C. 20231

Attn.: Assignment Branch

REQUEST FOR RECORDATION  
OF BLANKET ASSIGNMENT

Sir:

Enclosed herewith please find an original blanket Assignment dated April 4, 1991 between Enzo Biochem, Inc. and Enzo Diagnostics, Inc. This document is being submitted pursuant to 37 CFR 1.331 for recording in the Assignment Division of the Patent and Trademark Office. Please record this document and return the recorded document to the undersigned.

The Patent and Trademark Office is hereby authorized to charge Deposit Account No. 05-1135 for the recordation fee in the amount of \$8.00. The Patent and Trademark Office is also hereby authorized to charge Deposit Account No. 05-1135 for any additional fees required in connection herewith and to credit any overpayment therein.

EXPRESS MAIL CERTIFICATE	
"Express Mail" Label No.	FB 283463026
Deposit Date	April 16, 1991
I hereby certify that this paper and the attachments herein are being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 CFR 1.10 on the date indicated above and is addressed to the Commissioner of Patents and Trademarks, Washington DC 20231.	
<i>Elaine P. Brenner</i>	4/16/91
Elaine P. Brenner	Date
Reg. No. 29,517	

Respectfully submitted,

*Elaine P. Brenner*

Elaine P. Brenner  
Registration No. 29,517

Enzo Diagnostics, Inc.

40 Oak Drive  
Syosset, NY 11791  
(212) 924-5409 or 9578

## ASSIGNMENT

In consideration for One Dollar (\$1.00) and other good and valuable consideration, the receipt of which is hereby acknowledged, Elazar Rabbani, the undersigned, on behalf of Enzo Blochem, Inc., the assignor hereunder, a corporation of the State of New York, having a place of business at 345 Hudson Street, New York, New York 10014;

Hereby sells, assigns and transfers to: Enzo Diagnostics, Inc., a corporation of the State of New York, having a place of business at 40 Oak Drive, Syosset, NY 11791, its successors, assigns and legal representatives, the entire right, title and interest for the United States and all foreign countries, in and to any and all improvements which are disclosed in the following applications for Letters Patent:

Serial No.: 140,980  
Title: "Modified Nucleotides, Methods of Preparing and Utilizing and Compositions Containing The Same"  
Inventors: Dean Engelhardt, et al.  
Filing Date: January 5, 1988  
(Doc. No.: Enz-5(C)2)

Serial No.: 539,279  
Title: "Modified Nucleotides, Methods of Preparing and Utilizing and Compositions Containing The Same"  
Inventors: Dean Engelhardt, et al.  
Filing Date: June 15, 1990  
Doc. No.: Enz-5(Div. 2)(C))

Serial No.: 520,682  
Title: "Modified Nucleotides, Methods of Preparing and Utilizing and Compositions Containing The Same"  
Inventors: Dean Engelhardt, et al.  
Filing Date: May 8, 1990  
(Doc. No.: Enz-5(Div. 3))

Serial No.: 532,704  
Title: "Modified Nucleotides, Methods of Preparing and Utilizing and Compositions Containing The Same"  
Inventors: Dean Engelhardt, et al.  
Filing Date: June 4, 1990  
(Doc. No.: Enz-5(Div. 4))

Enzo Diagnostics, Inc.

Serial No.: 531,953  
Title: "Modified Nucleotides, Methods of Preparing and Utilizing and Compositions Containing The Same"  
Inventors: Dean Engelhardt, et al.  
Filing Date: June 1, 1990  
(Doc. No.: Enz-5(Div. 5))

Serial No.: 532,461  
Title: "Modified Nucleotides, Methods of Preparing and Utilizing and Compositions Containing The Same"  
Inventors: Dean Engelhardt, et al.  
Filing Date: May 31, 1990  
(Doc. No.: Enz-5(Div. 6))

Serial No.: 567,039  
Title: "Modified Nucleotides, Methods of Preparing and Utilizing and Compositions Containing The Same"  
Inventors: Dean Engelhardt, et al.  
Filing Date: August 13, 1990  
(Doc. No.: Enz-5(Div. 7))

Serial No.: 548,348  
Title: "Modified Nucleotides, Methods of Preparing and Utilizing and Compositions Containing The Same"  
Inventors: Dean Engelhardt, et al.  
Filing Date: July 2, 1990  
(Doc. No.: Enz-5(Div. 8))

Serial No.: 607,347  
Title: "Methods and Structures Employing Chemically Labeled Polynucleotide Probes"  
Inventors: Jannis Stavrianopoulos, et al.  
Filing Date: October 30, 1990  
(Doc. No.: Enz-7(CIP)C2)

Serial No.: 120,205  
Title: "Kit for Terminally Labeling DNA"  
Inventors: Christine Brakel, et al.  
Filing Date: November 5, 1987  
(Doc. No.: Enz-8(C)2)

Serial No.: 607,787  
Title: "Assay Method Utilizing Polynucleotide Sequences"  
Inventors: Robert Pergolizzi, et al.  
Filing Date: October 26, 1990  
(Doc. No.: Enz-11 (C)2)

Enzo Diagnostics, Inc.

Serial No.: 521,762  
Title: "Detectable Molecules, Method of Preparation and Use"  
Inventors: Dean Engelhardt, et al.  
Filing Date: May 8, 1990  
(Doc. No.: Enz-13 (Div. 4)C2)

Serial No.: 428,087  
Title: "Hybridization Method For The Detection Of Genetic Material"  
Inventors: Dean Engelhardt, et al.  
Filing Date: October 27, 1989  
(Doc. No.: Enz-15(Div. 1))

Serial No.: 590,548  
Title: "In Vivo Labelling of Polynucleotide Sequences"  
Inventors: Jannis Stavrianopoulos, et al.  
Filing Date: September 27, 1990  
(Doc. No.: Enz-16(C) (Div. 1))

Serial No.: 502,199  
Title: "Method For Detecting An Analyte Moiety By Means of Signal Localization"  
Inventor: Elazar Rabbani  
Filing Date: March 28, 1990  
(Doc. No.: Enz-24(CIP)/25 C1)

Serial No.: 501,643  
Title: "Method For Detecting An Analyte Moiety By Means of Signal Localization"  
Inventor: Elazar Rabbani  
Filing Date: March 29, 1990  
(Doc. No.: Enz-24(CIP)/25 Div.1)

Serial No.: 394,284  
Title: "Novel One Step Method and Polynucleotide Compounds For Hybridizing to Target Polynucleotides"  
Inventors: Jannis Stavrianopoulos, et al.  
Filing Date: August 15, 1989  
(Doc. No.: Enz-26(C))

Serial No.: 479,327  
Title: "Nucleotide Sequence Composition And Method For Detection of Neisseria Gonorrhoea And Method For Screening For A Nucleotide Sequence That Is Specific For A Genetically Distinct Group"

Inventors: Andrew Lo, et al.  
Filing Date: February 13, 1990  
(Doc. No.: Enz-27(C))

Serial No.: 582,830  
Title: "Polynucleotide Probes And A Method For Their Preparation"

Inventors: David Mao, et al.  
Filing Date: September 13, 1990  
(Doc. No.: Enz-29(C))

Serial No.: 509,110  
Title: "Polynucleotide Composition and Method"  
Inventors: Huey-Lang Yang, et al.  
Filing Date: April 13, 1990  
(Doc. No.: Enz-31(C))

Serial No.: 443,812  
Title: "Inhibition of Self-Quenching In Specific Binding Assay"  
Inventor: Stanley Kline  
Filing Date: November 29, 1989  
(Doc. No.: Enz-32(C))

Serial No.: 160,607  
Title: "End-Labeled Nucleotide Probe"  
Inventors: Christine L. Brakel, et al.  
Filing Date: February 26, 1988  
(Doc. No.: Enz-34)

Serial No.: 194,702  
Title: "Device With Gradient-Regulated Localized Signal"  
Inventors: Elazar Rabbani, et al.  
Filing Date: May 17, 1988  
(Doc. No.: Enz-35)

Serial No.: 192,256  
Title: "Method For Sample Adherence To Test Substrate"  
Inventors: Huey-Lang Yang, et al.  
Filing Date: May 10, 1988  
(Doc. No.: Enz-36)

Serial No.: 625,584  
Title: "Detection System For Visualizing Proteolysis Of Cellular Proteins"  
Inventor: Maurice Rosenstrauss  
Filing Date: December 10, 1990  
(Doc. No.: Enz-37(C))

Serial No.: 304,748  
Title: "Time-Resolved Fluorometer"  
Inventor: Ian Hurley  
Filing Date: January 31, 1989  
(Doc. No.: Enz-38)

Serial No.: 314,995  
Title: "Energy Transfer Hybridization Assay Using Intercalators and Lanthanide Metals"  
Inventors: Elazar Rabbani, et al.  
Filing Date: February 24, 1989  
(Doc. No.: Enz-39)

Serial No.: 342,196  
Title: "Non-Radioactive In Situ Hybridization Assay"  
Inventors: Joanne Spadaro, et al.  
Filing Date: April 21, 1989  
(Doc. No.: Enz-40)

Serial No.: 316,385  
Title: "Dual-Probe Energy Transfer Hybridization Assay"  
Inventors: Ian Hurley, et al.  
Filing Date: February 27, 1989  
(Doc. No.: Enz-41)

Serial No.: 459,030  
Title: "Amplification Capture Assay"  
Inventors: Christine Brakel, et al.  
Filing Date: December 29, 1989  
(Doc. No.: Enz-43)

Serial No.: 633,730  
Title: "Method for Detecting A Target  
Polynucleotide In A Sample Using  
A Background Reducing Reagent, And  
Composition And Kit Comprising Such  
A Reagent"  
Inventor: Jannis G. Stavrianopoulos  
Filing Date: December 24, 1990  
(Doc. No.: Enz-44)

and in and to said applications for Letters Patent and all divisional, continuing, substitute, renewal, reissue, and all other applications for Letters Patent which have been or shall be filed in the United States and all foreign countries on any of said improvements; and in and to all original and reissued patents which have been or shall be issued in the United States and all foreign countries on said improvements:

Hereby also sells, assigns and transfers to: Enzo Diagnostics, Inc., a corporation of the State of New York, having a place of business at 40 Oak Drive, Syosset, NY 11791, its successors, assigns and legal representatives, the entire right, title and interest for the United States and all foreign countries, in and to any and all improvements which are disclosed in the following United States Letters Patent:

Patent No.: 4,994,373  
Title: "Method And Structures Employing  
Chemically-Labelled Polynucleotide Probes"  
Inventors: Jannis G. Stavrianopoulos, et al.  
Issue Date: February 19, 1991  
(Doc. No.: Enz-7 (CIP)C)

Patent No.: 4,772,548  
Title: "Radioisotopicassay Using Isotope Transfer  
To Chelator-Target Recognition Molecule  
Conjugate"  
Inventor: Jannis G. Stavrianopoulos  
Issue Date: September 20, 1988  
(Doc. No.: Enz-12 (Div. 2))

Patent No.: 4,707,440  
Title: "Nucleic Acid Hybridization Assay  
And Detectable Molecules Useful In  
Such Assay"  
Inventor: Jannis G. Stavrianopoulos  
Issue Date: November 17, 1987  
(Doc. No.: Enz-13)

Enzo Diagnostics, Inc.



Patent No.: 4,843,122  
Title: "Detectable Molecules, Method  
Of Preparation And Use"  
Inventor: Jannis G. Stravrianopoulos  
Issue Date: June 27, 1989  
(Doc. No.: Enz-13 (Div. 1))

Patent No.: 4,943,523  
Title: "Detectable Molecules, Method  
Of Preparation And Use"  
Inventor: Jannis G. Stravrianopoulos  
Issue Date: July 24, 1990  
(Doc. No.: Enz-13 (Div. 2))

Patent No.: 4,952,685  
Title: "Detectable Molecules, Method  
Of Preparation And Use"  
Inventor: Jannis G. Stravrianopoulos  
Issue Date: August 28, 1990  
(Doc. No.: Enz-13 (Div. 4))

Patent No.: 5,002,885  
Title: "Detectable Molecules, Method  
Of Preparation And Use"  
Inventor: Jannis G. Stravrianopoulos  
Issue Date: March 26, 1991  
(Doc. No.: Enz-13 (Div. 4)C1)

Patent No.: 4,849,505  
Title: "Detectable Molecules, Method  
Of Preparation And Use"  
Inventor: Jannis G. Stravrianopoulos  
Issue Date: July 18, 1989  
(Doc. No.: Enz-13 (Div. 5))

Patent No.: 4,889,798  
Title: "Heterologous System For The  
Detection of Chemically Labeled  
DNA and Other Biological Materials  
Providing A Receptor Or Target  
Moiety Thereon"  
Inventor: Elazar Rabbani  
Issue Date: December 26, 1989  
(Doc. No.: Enz-14(C))


Patent No.:	4,894,325
Title:	"Hybridization Method For The Detection Of Genetic Material"
Inventors:	Dean L. Engelhardt, et al.
Issue Date:	January 16, 1990
(Doc. No.:	Enz-15(P)C)
Patent No.:	4,987,065
Title:	"In Vivo Labelling of Polynucleotide Sequences"
Inventors:	Jannis G. Stravrianopoulos, et al.
Issue Date:	January 22, 1991
(Doc. No.:	Enz-16(C))
Patent No.:	4,755,458
Title:	"Composition And Method For The Detection Of The Presence Of A Polynucleotide Sequence Of Interest"
Inventors:	Elazar Rabbani, et al.
Issue Date:	July 5, 1988
(Doc. No.:	Enz-18)
Patent No.:	4,746,604
Title:	"Specific Binding Assays Utilizing A Viable Cell As A Label"
Inventor:	Solomon Mowshowitz
Issue Date:	May 24, 1988
(Doc. No.:	Enz-21)
Patent No.:	4,900,659
Title:	"Nucleotide Sequence Composition And Method For Detection Of Neisseria Gonorrhoeae And Method For Screening For A Nucleotide Sequence That Is Specific For A Genetically Distinct Group"
Inventors:	Andrew Lo, et al.
Issue Date:	February 13, 1990
(Doc. No.:	Enz-27)
Patent No.:	4,868,103
Title:	"Analyte Detection By Means Of Energy Transfer"
Inventors:	Jannis G. Stravrianopoulos, et al.
Issue Date:	September 19, 1989
(Doc. No.:	Enz-28)

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Enzo Diagnostics, Inc.

Patent which have been or shall be filed in the United States and all foreign countries on any of said improvements; and in and to all original and reissued patents which have been or shall be issued in the United States and all foreign countries on said improvements:

Agrees that said Assignee may apply for and receive Letters Patent for said improvements in its own name; and that, when requested, without charge to but at the expense of said Assignee, its successors, assigns and legal representatives, to carry out in good faith the intent and purpose of this assignment, the undersigned will execute all divisional, continuing, substitute, renewal, reissue, and all other patent applications on any and all said improvements; execute all rightful oaths, assignments, powers of attorney and other papers, communicate to said Assignee, its successors, assigns, and representatives, all facts known to the undersigned relating to said improvements and the history thereof; and generally do everything possible which said Assignee, its successors, assigns or representatives shall consider desirable for aiding in securing and maintaining proper patent protection for said improvements and for vesting title to said improvements and all applications for Letters Patent and all patents on said improvements, in said Assignee, its successors, assigns and legal representatives; and

Covenant with said Assignee, its successors, assigns and legal representatives that no assignment, grant, mortgage, license or other agreement affecting the rights and property herein conveyed has been made to others by the undersigned, and that full right to convey the same as herein expressed is possessed by the undersigned.

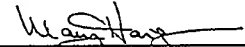
  
Elazar Rabbani, President  
Enzo Biochem, Inc.

#### Acknowledgment

State of NEW YORK }  
County of NEW YORK } ss:

On this 14<sup>th</sup> day of April, 1991 personally appeared before me ELAZAR RABBANI to me known, and known by me to be the same person described in and who executed the foregoing instrument, and acknowledged that he executed the same, of his own free will and for the purposes set forth.

MARY HAIG  
Notary Public, State of New York  
No. 41-1626625  
Qualified in Queens County  
Certificate Filed in New York County  
Commission Expires October 31, 1991

  
Notary Public

Enzo Diagnostics, Inc.

# **EXHIBIT 2**



Patent and Trademark Office  
ASSISTANT SECRETARY AND COMMISSIONER  
OF PATENTS AND TRADEMARKS  
Washington, D.C. 20231

TO: ENZO DIAGNOSTICS, INC.  
40 OAK DRIVE  
SYOSSET, NY 11791

RECEIVED  
SEP 05 1991  
ELAINE P. BRENNER

UNITED STATES PATENT AND TRADEMARK OFFICE  
NOTICE OF RECORDATION OF ASSIGNMENT DOCUMENT

THE ENCLOSED DOCUMENT HAS BEEN RECORDED BY THE ASSIGNMENT DIVISION OF THE U.S. PATENT AND TRADEMARK OFFICE. A COMPLETE MICROFILM COPY IS AVAILABLE AT THE U.S. PATENT AND TRADEMARK OFFICE ON THE REEL AND FRAME NUMBER REFERENCED BELOW. A DIGEST OF THE DOCUMENT HAS ALSO BEEN MADE AND APPEARS IN THE OFFICE'S RECORDS AS SHOWN:

ASSIGNOR: 001 ENZO BIOCHEM, INC., A CORP. OF NY      DOC DATE: 04/04/91

RECORDATION DATE: 04/17/91      NUMBER OF PAGES 010      REEL/FRAME 5673/0274

DIGEST: ASSIGNMENT OF ASSIGNORS INTEREST

ASSIGNEE: 501 ENZO DIAGNOSTICS, INC., 40 OAK DRIVE, SYOSSET, NY 11791, A CORP. OF NY

SERIAL NUMBER	6-575396	FILING DATE	01/30/84
PATENT NUMBER	4,707,440	ISSUE DATE	11/17/87

SERIAL NUMBER	7-043568	FILING DATE	04/28/87
PATENT NUMBER	4,843,122	ISSUE DATE	06/27/89

SERIAL NUMBER	7-304748	FILING DATE	01/31/89
PATENT NUMBER		ISSUE DATE	00/00/00

SERIAL NUMBER	7-314995	FILING DATE	02/24/89
PATENT NUMBER		ISSUE DATE	00/00/00

SERIAL NUMBER	7-342196	FILING DATE	04/21/89
PATENT NUMBER		ISSUE DATE	00/00/00

SERIAL NUMBER	7-316385	FILING DATE	02/27/89
PATENT NUMBER		ISSUE DATE	00/00/00

SERIAL NUMBER	7-459030	FILING DATE	12/29/89
PATENT NUMBER		ISSUE DATE	00/00/00

SERIAL NUMBER	7-633730	FILING DATE	12/24/90
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PATENT NUMBER	ISSUE DATE	00/00/00
SERIAL NUMBER 7-385986	FILING DATE 07/20/89	
PATENT NUMBER 4,994,373	ISSUE DATE 02/19/91	
SERIAL NUMBER 7-006819	FILING DATE 01/23/87	
PATENT NUMBER 4,772,548	ISSUE DATE 09/20/88	
SERIAL NUMBER 7-043670	FILING DATE 04/28/87	
PATENT NUMBER 4,943,523	ISSUE DATE 07/24/90	
SERIAL NUMBER 7-043572	FILING DATE 04/28/87	
PATENT NUMBER 4,849,505	ISSUE DATE 07/18/89	
SERIAL NUMBER 7-015563	FILING DATE 02/13/87	
PATENT NUMBER 4,889,798	ISSUE DATE 12/26/89	
SERIAL NUMBER 7-005327	FILING DATE 01/15/87	
PATENT NUMBER 4,894,325	ISSUE DATE 01/16/90	
SERIAL NUMBER 7-302887	FILING DATE 01/30/89	
PATENT NUMBER 4,897,065	ISSUE DATE 01/30/90	
SERIAL NUMBER 6-646171	FILING DATE 08/30/84	
PATENT NUMBER 4,755,458	ISSUE DATE 07/05/88	
SERIAL NUMBER 6-738135	FILING DATE 05/24/85	
PATENT NUMBER 4,746,604	ISSUE DATE 05/24/88	
SERIAL NUMBER 6-823921	FILING DATE 01/30/86	
PATENT NUMBER 4,900,659	ISSUE DATE 02/13/90	
SERIAL NUMBER 6-831250	FILING DATE 02/19/86	
PATENT NUMBER 4,868,103	ISSUE DATE 09/19/89	
SERIAL NUMBER 7-194702	FILING DATE 05/17/88	
PATENT NUMBER	ISSUE DATE 00/00/00	
SERIAL NUMBER 7-192256	FILING DATE 05/10/88	
PATENT NUMBER 5,024,933	ISSUE DATE 06/18/91	
SERIAL NUMBER 7-625584	FILING DATE 12/10/90	
PATENT NUMBER	ISSUE DATE 00/00/00	
SERIAL NUMBER 7-140980	FILING DATE 01/05/88	
PATENT NUMBER	ISSUE DATE 00/00/00	Ann-5(c)2
SERIAL NUMBER 7-539279	FILING DATE 06/15/90	
PATENT NUMBER	ISSUE DATE 00/00/00	
SERIAL NUMBER 7-520682	FILING DATE 05/08/90	
PATENT NUMBER	ISSUE DATE 00/00/00	

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SEP 05 1991

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SERIAL NUMBER	7-531953	FILING DATE	06/01/90
PATENT NUMBER		ISSUE DATE	00/00/00
SERIAL NUMBER	7-532461	FILING DATE	05/31/90
PATENT NUMBER		ISSUE DATE	00/00/00
SERIAL NUMBER	7-567039	FILING DATE	08/13/90
PATENT NUMBER		ISSUE DATE	00/00/00
SERIAL NUMBER	7-532704	FILING DATE	06/04/90
PATENT NUMBER		ISSUE DATE	00/00/00
SERIAL NUMBER	7-548348	FILING DATE	07/02/90
PATENT NUMBER		ISSUE DATE	00/00/00
- SERIAL NUMBER	7-607347	FILING DATE	10/30/90
PATENT NUMBER		ISSUE DATE	00/00/00
SERIAL NUMBER	7-120205	FILING DATE	11/05/87
PATENT NUMBER		ISSUE DATE	00/00/00
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SERIAL NUMBER	7-443812	FILING DATE	11/29/89
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SERIAL NUMBER	7-479327	FILING DATE	02/13/90
PATENT NUMBER		ISSUE DATE	00/00/00
- SERIAL NUMBER	7-582830	FILING DATE	09/13/90
PATENT NUMBER		ISSUE DATE	00/00/00
SERIAL NUMBER	7-590548	FILING DATE	09/27/90
PATENT NUMBER		ISSUE DATE	00/00/00
SERIAL NUMBER	7-502199	FILING DATE	03/28/90
PATENT NUMBER		ISSUE DATE	00/00/00
SERIAL NUMBER	7-501643	FILING DATE	03/29/90
PATENT NUMBER		ISSUE DATE	00/00/00
SERIAL NUMBER	7-607787	FILING DATE	10/26/90
PATENT NUMBER		ISSUE DATE	00/00/00

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SEP 06 1991

ELIANE P. BRENNER

CONTINUED 5673/0274



Patent and Trademark Office  
ASSISTANT SECRETARY AND COMMISSIONER  
OF PATENTS AND TRADEMARKS  
Washington, D.C. 20231

SERIAL NUMBER 7-521762  
PATENT NUMBER 5,013,831

FILING DATE 09/08/90  
ISSUE DATE 05/07/91

SERIAL NUMBER 7-428087  
PATENT NUMBER

FILING DATE 10/27/89  
ISSUE DATE 00/00/00

SERIAL NUMBER 7-043668  
PATENT NUMBER 4,952,685

FILING DATE 04/28/87  
ISSUE DATE 08/28/90

SERIAL NUMBER 7-513723  
PATENT NUMBER 5,002,885

FILING DATE 04/24/90  
ISSUE DATE 03/26/91

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SEP 05 1991

ELAINE P. BRENNER



# EXHIBIT 3

## Assignment

In consideration of One Dollar (\$1.00), and other good and valuable considerations, the receipt of which is hereby acknowledged, we, the undersigned, Dean Engelhardt, Elazar Rabbani, Stanley Kline, ~~James G.~~ <sup>James G.</sup> Stavrianopoulos and Dollie Kirtikar, residing at 173 Riverside Drive, New York, NY 10024, 69 Fifth Avenue, New York, NY 10003, 235 Lincoln Place, Brooklyn, NY 11217, 515 West 59 Street, New York, NY 10019 and 42-72 80 Street, Elmhurst, NY 11373, respectively,

Hereby sell, assign and transfer to Enzo Biochem, Inc.

a corporation of the State of New York having a place of business at 325 Hudson Street, New York, N.Y. 10013 in the County of New York and State of New York, its successors, assigns and legal representatives, the entire right, title and interest for all countries, in and to any and all inventions which are disclosed and claimed, and any and all inventions which are disclosed but not claimed, in the application for United States Patent, which has been executed by the undersigned on June 22, 1982 and is entitled

MODIFIED NUCLEOTIDES, METHODS OF PREPARING AND UTILIZING  
AND COMPOSITIONS CONTAINING THE SAME

and in and to said application and all divisional, continuing, substitute, renewal, reissue, and all other applications for U.S. Letters Patent or other related property rights in any and all foreign countries which have been or shall be filed on any of said inventions disclosed in said application; and in and to all original and reissued patents or related foreign documents which have been or shall be issued on said inventions;

Authorize and request the Commissioner of Patents of the United States to issue to said Assignee, the corporation above named, its successors, assigns and legal representatives, in accordance with this assignment, any and all United States Letters Patent on said inventions or any of them disclosed in said application;

NEEL 4019 FRAME 405

Agree that said Assignee may apply for and receive foreign Letters Patent or rights of any other kind for said inventions, or any of them; and may claim, in applications for said foreign Letters Patent or other rights, the priority of the aforesaid United States patent application under the provisions of the International Convention of 1883 and later modifications thereof, under the Patent Cooperation Treaty, under the European Patent Convention or under any other available international agreement; and that, when requested, without charge to, but at the expense of, said Assignee, its successors, assigns and legal representatives, to carry out in good faith the intent and purpose of this assignment, the undersigned or the undersigned's executors or administrators will, for the United States and all foreign countries, execute all divisional, continuing, substitute, renewal, reissue, and all other patent applications or other documents on any and all said inventions; execute all rightful oaths, assignments, powers of attorney and other papers; communicate to said Assignee, its successors, assigns and representatives, all facts known and documents available to the undersigned relating to said inventions and the history thereof; testify in all legal proceedings; and generally do everything possible which said Assignee, its successors, assigns or representatives shall consider desirable for aiding in securing, maintaining and enforcing proper patent protection for said inventions and for vesting title to said inventions and all applications for patents or related foreign rights and all patents on said inventions, in said Assignee, its successors, assigns and legal representatives; and

**Covenant** with said Assignee, its successors, assigns and legal representatives that no assignment, grant, mortgage, license or other agreement affecting the rights and property herein conveyed has been made to others by the undersigned, and that full right to convey the same as herein expressed is possessed by the undersigned.

Date: June 22 1982  
Witness: Thomas F. Moran

Dean Engelhardt [L.S.]  
Dean Engelhardt

Date: JUN 22 1982  
Witness: Thomas F. Moran

Elazar Rabbani [L.S.]  
Elazar Rabbani

REC-4019 FROM 406

Stanley Kline [L.S.]

Date: June 22, 1982

Stanley Kline

Witness: \_\_\_\_\_

Thomas J. Moran

\_\_\_\_\_

\_\_\_\_\_

James G. Stavrianopoulos [L.S.]  
JAMES G. Stavrianopoulos

Date: June 22 1982 19

Witness: Thomas J. Moran

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Dollie Kirtikar [L.S.]

Date: June 22nd 1982 19

Dollie Kirtikar

Witness: Thomas J. Moran

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RECORDED  
PATENT & TRADEMARK OFFICE

JUN 23 1982

James G. Stavrianopoulos  
COMMISSIONER OF PATENTS  
AND TRADEMARKS

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